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Electrochemical Detection of Gel Microparticles in Seawater*

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We present the first atomic force microscopy (AFM) images of the native marine gel network and a new type of electrochemical signals of individual gel microparticles in seawater. Gel microparticles in seawater are selectively detected through specific amperometric signals using a dropping mercury electrode (DME) as a sensor. We have demonstrated that organic microparticles naturally present in Northern Adriatic seawater can be detected as single particles and sorted at the DME/seawater interface according to their hydrophobicity and supramolecular organization.

INTRODUCTION

Systematic studies focused on the easily perturbed three-dimensional structure of marine particles[1] are rare due to the lack of appropriate techniques. Knowledge about the structure of marine colloidal particles[2] was mostly acquired by transmission electron microscopy (TEM)[2–6] and only more recently by atomic force microscopy AFM[3,7] and environmental scanning electron microscopy (ESEM).[8] AFM in the non-contact mode[9–11] has allowed hydrated polymer gels to be observed by unprecedented clarity.[12] Each of these techniques requires various steps of pretreatment prior to the observation, which may bias the results. These instruments and techniques are not suitable for routine analysis or monitoring, although they could provide a valuable insight into the macromolecular organization. On the other hand, electroanalytical techniques offer an important and convenient variety of tools for field and *in situ* analysis. Their efficiencies, their sensitivities, and the easy way they are operated by non-specialists, as well as their relatively low cost, make these techniques increasingly attractive.[13,14]

The dropping mercury electrode (DME)[15] immersed directly in fresh unfiltered seawater samples has been already used as an *in situ* sensor to characterize micrometer sized surface-active particles.[16–18] Surface-active particles have been described as fluid vesicle-like structures formed by self-organization of polysaccharide, proteinaceous and lipid components of the excretion and decomposition products of phytoplankton.[1,17]

It has to be pointed out that the dropping mercury electrode has a fast growing renewable surface and the analysis can be repeated many times at will. This is an important aspect of the method since the arrival of parti-

* Dedicated to the memory of the late Professor Marko Branica

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cles to the interface is a stochastic process and the representative behavior can be determined only by analyzing a larger set of data collected under an identical experimental environment.[19]

The method is basically chronoamperometry of dissolved oxygen at potentials of the current maximum (»polarographic maximum«)[20–22] caused by gradients of interfacial tension at the expanding mercury drop/seawater interface. Adsorption of organic molecules causes a decrease of the surface tension gradients at the mercury electrode/solution interface and consequently suppression of convective streaming. On the chronoamperometric curve, adsorption is manifested as a gradual decrease of the oxygen reduction current.[20,23] On the other hand, surface-active particles can be characterized through their adhesion signals in the same way as organic droplets or cells.[22–26] The signal of a surface-active particle appears as a sharp perturbation on the current-time (*I-t*) curves of oxygen reduction due to a localized drop of interfacial tension caused by molecular contact. The amplitude of signals reflects the size and the frequency of signals reflects the concentration of particles.

Here, we explore electrochemical detection of marine gel microparticles using the DME as a sensor. AFM is introduced to image 3-D structure of the gel matrix. The current-time curves of oxygen reduction at DME exhibit specific perturbations in the form of narrow depressions. This type of amperometric signals has not been previously described in the electrochemical literature.

The mere possibility of direct and specific detection of gel microparticles in seawater would be of the utmost importance for understanding the organization and transformation processes of organic matter in the sea.[1,27]

EXPERIMENTAL

Native Gel Phase

Model gel particles were prepared in the laboratory using the natural gel phase that appears episodically in the Northern Adriatic[28,29] as large aggregates, »mucilage« within the water column (Figure 1).

Current views[1,29,30] leave no doubt about phytoplankton production of polysaccharides[31,32] as the main organic components of the macrogels. During the episode of massive macroaggregate formation in the Northern Adriatic that started in the late spring of 2000, we collected material of a large ($d > 1$ m) aggregate residing at a 10-m depth (station SJ007, 13°16'E, 45°17'N, sampling date June 30). Salinity of the surrounding seawater was 36.92 ‰ and the temperature was 23.25 °C. Sampling was done by scuba-divers using 1000 mL plastic cylinders. Aggregate samples were stored in dark glass bottles and within 24 hours transported to the laboratory where the basic physicochemical properties were determined first. The values at 20 °C are: specific density 1.0167 g/cm^3, viscosity 20.863 cP, pH 7.51, and conductivity 51.5 mS/cm. The organic carbon content in an aggregate sample generally varied between 150 and 600 mg C/L.[33,34] Alciane blue assay[35] (micrograph shown in Figure 1) proved the predominance of acidic polysaccharides. The fresh sample was centrifuged (10 minutes at 10000 *g*) to eliminate excess seawater. 1000 mL of the initial sample yielded 200 mL of the gel phase. The gel was then rinsed from excess salts with ultra pure water and again separated by centrifugation. This material, native gel, was further used as the source material in preparation of gel microparticles and for AFM measurements.

AFM Imaging

An aliquot of native gel was placed in demineralized water (Milli-Q Plus) at a ratio of 1:30 or 1:100 and equilibrated for 24 hours under stirring prior to AFM specimen preparation. 5 μL of diluted gel was pipetted directly onto freshly cleaved mica. Following deposition, the mica sheets were placed into closed Petri dishes for approximately 20 minutes at a relative humidity of 60 % in order to evaporate the excess of water on mica. AFM imaging was performed on a Nanoscope III multimode scanning probe microscope (Digital Instruments) using a 10 μm × 10 μm piezoelectric scanner. Images were collected using the tapping mode AFM,

Figure 1. Northern Adriatic giant gel aggregates at 10 m depth captured by a scuba-diver in August 1997 (courtesy of Gerald Müller-Niklas). Insert shows a microphotograph of gel aggregate material stained with Alcian blue (bar denotes 10 μm).

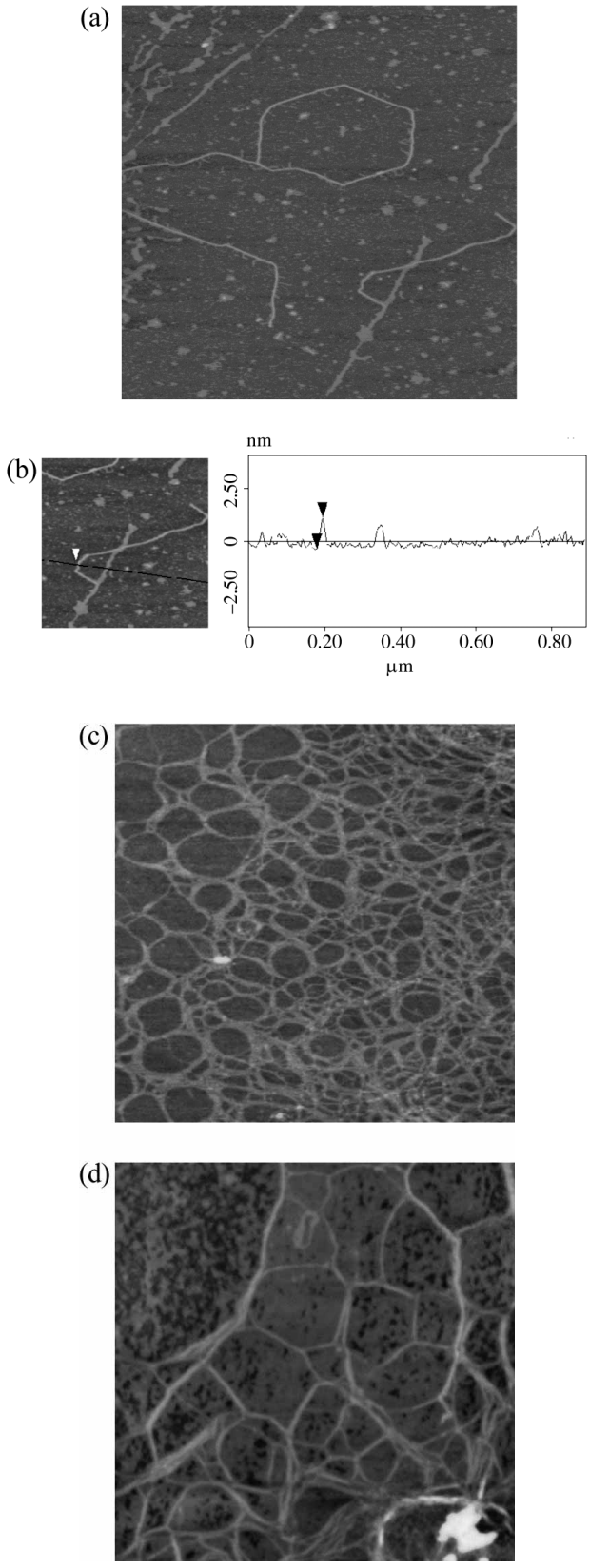

Figure 2. AFM images of native-gel polysaccharide chains (a,b) and of gel network (c,d). Tapping mode AFM image of diluted gel samples deposited on mica: (a) dilution 1:100, scan size 1.7 μm × 1.7 μm; (b) profile analysis of a fibril, height between the two cursors 1.3 nm; (c) dilution 1:30, scan size 2.0 μm × 2.0 μm; (d) dilution 1:100, scan size 2.0 μm × 2.0 μm.

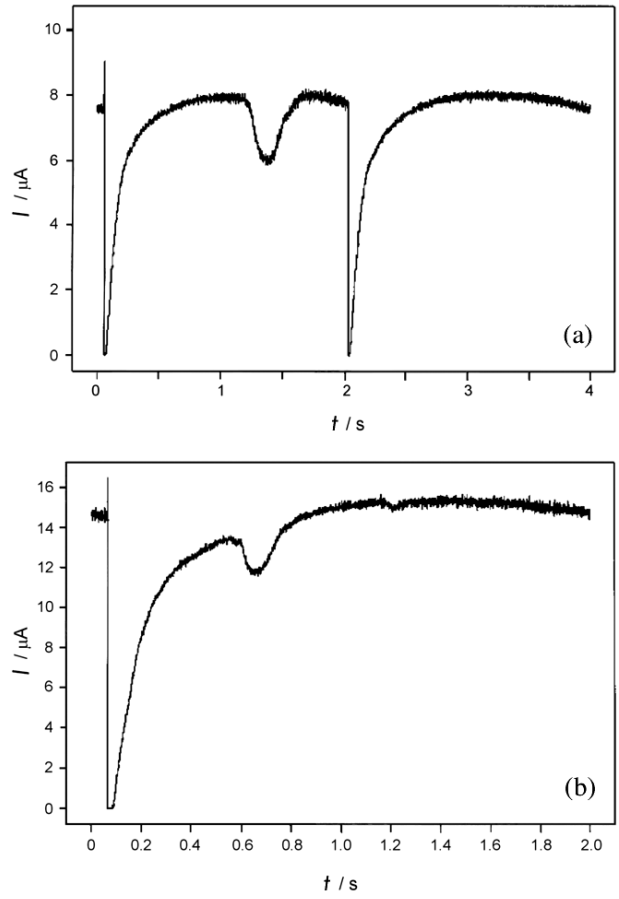

Figure 3. Electrochemical response of gel microparticles dispersion in seawater (400 μL of gel dispersed in 20 mL seawater): (a) two consecutive *I-t* curves recorded at 500 μs per point; (b) a single *I-t* curve recorded at 100 μs per point resolution.

which is particularly well adapted to soft samples due to a nearly complete reduction of lateral forces.[36] Silicon tips with a spring constant of 42 N m^{-1} and resonance frequency of approximately 320 kHz were used in the tapping mode. In order to minimize the interaction forces between tips and samples, the ratio of the set point amplitude to the tip free amplitude was maintained at 0.9 (»light tapping«). Images were recorded at 50–60 % relative humidity.

RESULTS

Figure 2 shows typical AFM images of the native gel sample on mica. The images demonstrate that an important fraction of the specimen consists of fibrillar materials. Some of the fibrils look like rigid macromolecules with typical diameters between 1–3 nm and lengths of 100 nm to 2 μm (Figures 2a,b). Although it is difficult to determine the nature of the fibril by simple observation, there is strong evidence that the fibrils are mainly polysaccharides, since AFM images of polysaccharide molecules have demonstrated similar features.[12,37–39] Furthermore, Alciane blue stained samples (insert in Figure 1) confirm the predominance of acidic polysaccharides.

Figures 2c,d are the first images of a native marine-gel network. The gel structure exhibits a repeating network of solvent cavities (ranging from 150 to 500 nm) between polymeric strands.

Electrochemical Detection of Gel Microparticles

The seawater sample (25–50 mL) was placed in an electrochemical cell open to air throughout the analysis and was thermostated at 20 °C. A dropping mercury electrode with the drop life of 2.0 seconds, flow rate 6 mg/s and the maximum surface area 4.57 mm^2 was used with Ag/AgCl electrode as a reference in the three-electrode configuration**.** Electrochemical measurements were performed with a PAR 174A Polarographic Analyzer. Constant potential of –400 mV was applied. At this potential, the dropping mercury electrode is positively charged (+3.8 $\mu C/cm^2$). Current-time curves were recorded with 50–200 μs per point time resolution and stored with a Nicolet 3091 digital oscilloscope connected to a PC.

*Electrochemical Signals of Gel Microparticles***. –** A volume fraction of 400 μL of native gel was dispersed in 20 mL of raw seawater by vortexing for 15 seconds. The number of gel particles in this dispersion and in the size fraction from 10 to 300 μm was determined by a Coulter counter to be 10.8×10^6/L. The blank experiment run with seawater used for dispersion preparation gave a total particle concentration of 2×10^5/L.

When the DME was immersed in the gel microparticles dispersion, the current-time curves of oxygen reduction exhibited specific, previously uncharacterized perturbations in the form of narrow depressions of oxygen reduction current (Figure 3) (duration 50–500 ms and amplitude 0.1–2.0 μA). In a series of consecutive *I-t* curves (one *I-t* curve corresponds to the DME life-time of 2 s), depression signals of varying amplitudes appeared at irregular intervals reflecting a random arrival of particles at the DME. The mean frequency of appearance of individual signals was 24 dips per 50 *I-t* curves, which corresponds to 6.9×10^6 particles/L. Since this result is close to the Coulter Counter value of 10.8×10^6 particles/L, it is concluded that each depression signal is caused by a single gel particle-electrode interaction.

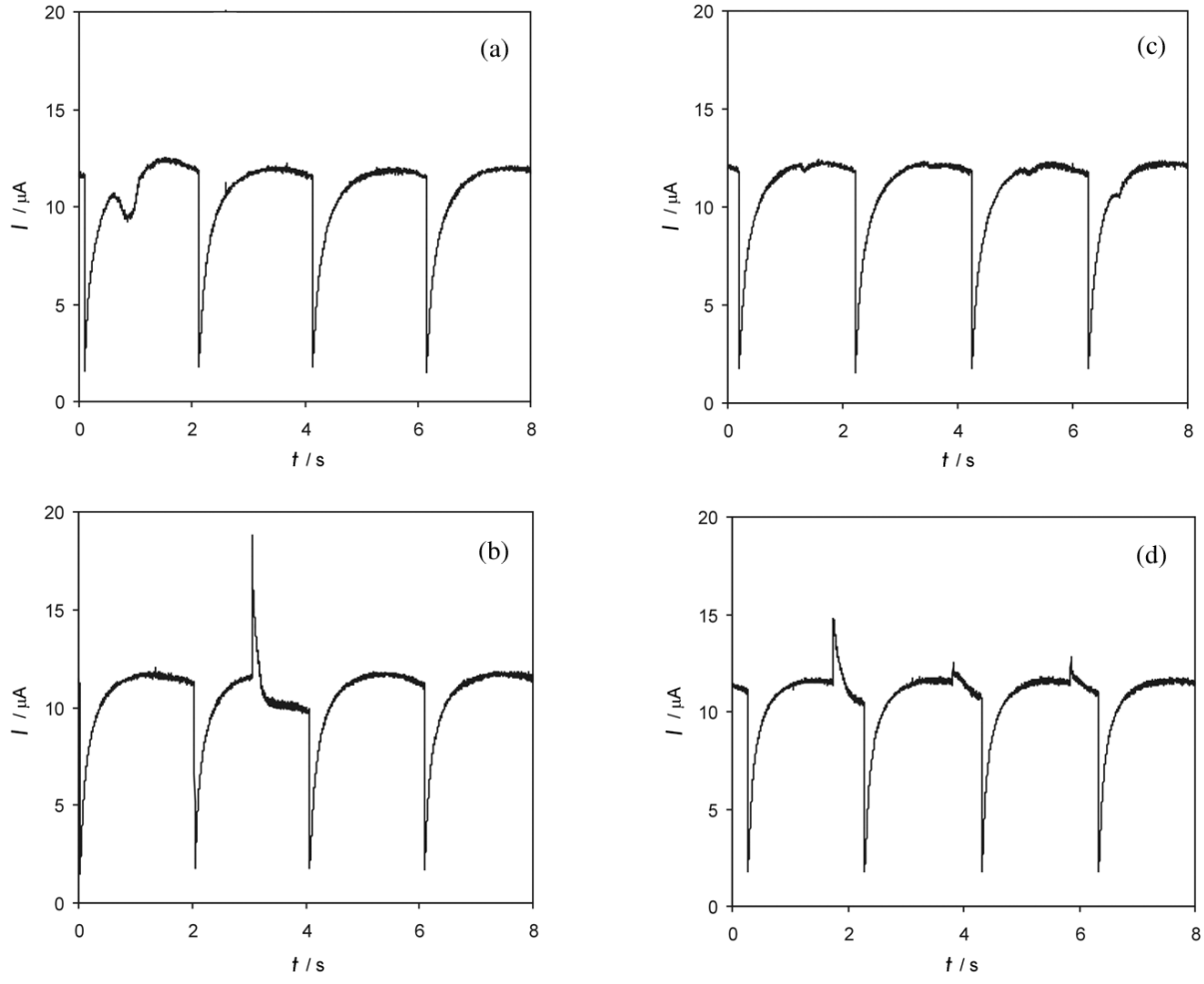

Figure 4. Electrochemical response of microparticles (actual recordings over four consecutive DME life-times) in seawater samples taken in the Northern Adriatic waters on November 28, 1998: (a) example of a pronounced depression signal in a series of four *I-t* curves; (b) example of a spike-shaped signal; (c) series of depression signals; (d) series of spike-shaped signals.

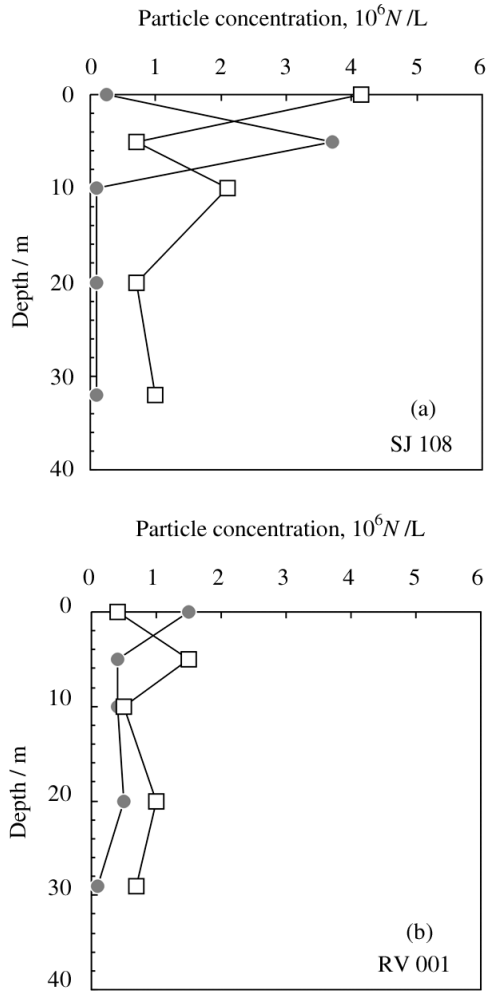

Figure 5. Vertical profiles of gel (□) and surface-active (●) microparticle concentrations determined by electrochemical analysis of seawater samples taken on October 28, 1998, at two sampling stations in the Northern Adriatic: (a) SJ108 (12°45′E, 44°45.4′N), close to the river Po estuary and (b) RV001 (13°36.6′E, 45°04.8′N) near the Istrian coast.

The shape of perturbations is exemplified with two *I-t* curves in Figure 3a: a pronounced depression signal appears in the first *I-t* curve, perturbing the smoothly growing oxygen reduction current represented by the second *I-t* curve (baseline current). Figure 3b shows an *I-t* curve, recorded at higher resolution in a separate set of experiments, where two consecutive depression signals having different amplitudes appear. After a depression signal there is no decrease of the baseline current (otherwise a typical feature of adhesion signals)[14,16,18,19] indicating that no permanent contact between the electrode and the gel particle was established.

The depression signal in the amperometric curve (Figure 3) reflects a transient decrease of dissolved oxygen supply at a fraction of the expanding DME interface affected by the collision of the gel microparticle with the growing mercury drop surface. This interpretation of depression signals is further supported by the fact that the gel microparticles could not be detected by recording amperometric curves of oxygen reduction using a static mercury electrode, or using DME in deaerated seawater. The empirical finding that electrical signals of gel particles (Figure 3) do not exhibit a sharp increase or a final decrease of the baseline-current of oxygen reduction signifies that the collision of gel microparticles did not cause any displacement of counter ions at the electrode/seawater interface. Hence, no intimate contact between the gel microparticle and the mercury surface was established. The lack of adhesion can be explained by the fact that biopolymer entanglement within the gel phase is stronger than the gel interaction with the electrode surface.

Gel Microparticles in the Northern Adriatic

In 1998, we started monitoring marine microparticles in the Northern Adriatic as possible precursors of »mucilage events«. In the seawater samples collected in October 1998, we observed a high incidence of pronounced depression signals. An example of the signals recorded in seawater samples in October 28, 1998 is shown in Figure 4. The number of spikes and depressions was determined for each sample in a series of 50 *I-t* curves (50 mercury drop-lives). The frequency of signals is translated into particle concentration using the calibration curve. Characteristic depths profiles for abundance of each particle class are shown in Figure 5. Upon analysis of a large set of monitoring data, we have established that gel microparticles in the Northern Adriatic seawater do not exceed 10 % of electrochemically detectable particles. At the onset of »mucilage events« (in summer 1997 and spring 2000), the concentration of surface-active particles attained a maximum value of 3×10^7/L, while gel microparticles could not be detected. This finding suggests that the macroscopic gel phase shown in Figure 1 was not formed by coalescence of pre-existing gel microparticles but through the phase transition of surface-active particles.[1,30]

DISCUSSION

Herein we have identified a new type of electrochemical signals (Figure 3), which we assign to the distinct class of marine microparticles of gel structure. We put forward the interpretation that collision of gel microparticles at the DME proceeds at a distance larger than the thickness of the inner Helmholtz plane (0.3 nm), without permanent attachment and spreading of the gel particle. The lack of adhesion can be explained by the fact that biopolymer entanglement within the gel phase is stronger than the gel interaction with the electrode surface.

Based on the phenomena of molecular adsorption or particle collision and adhesion at the DME, the organic constituents of seawater can be classified by their elec-

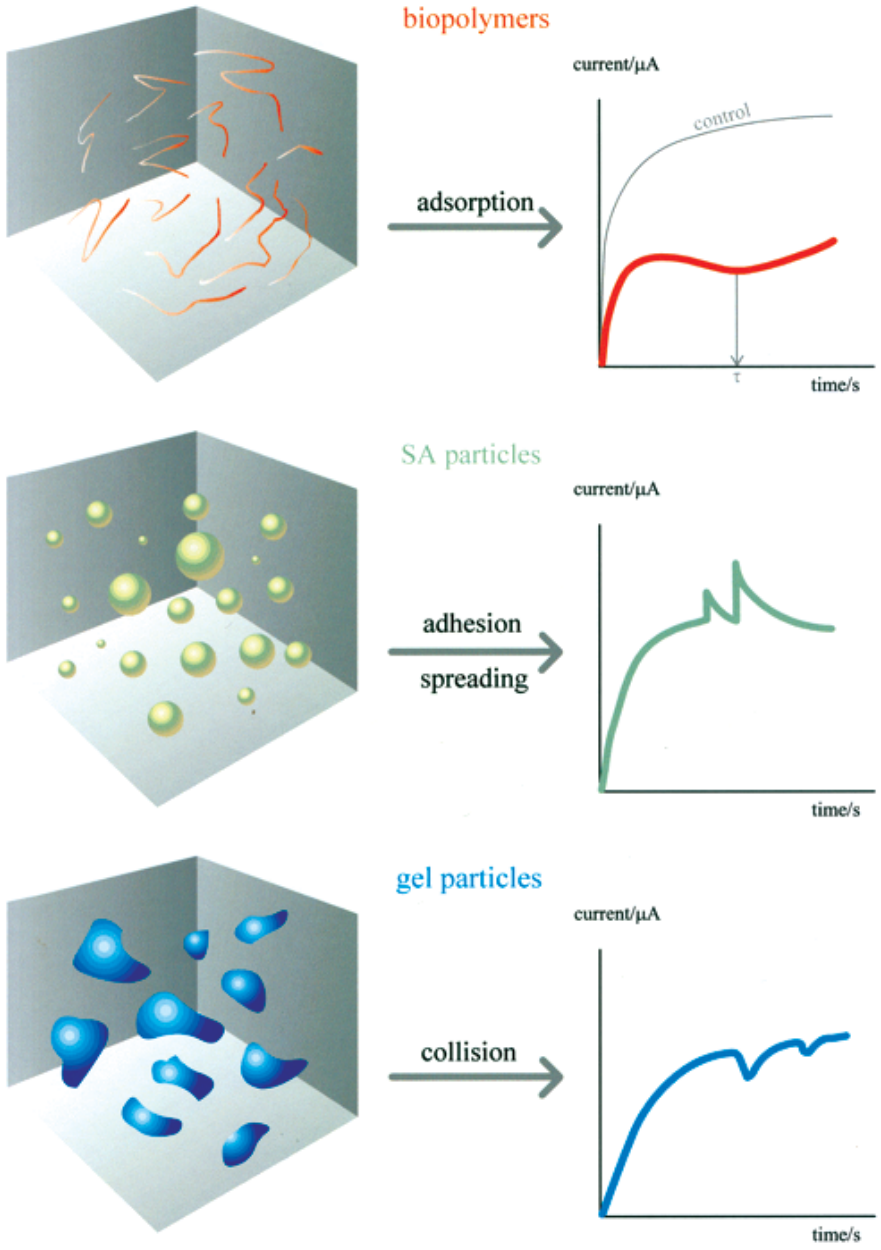

Figure 6. Illustration of different classes of organic constituents in seawater and their electrochemical signals at DME: biopolymers and small colloids, surface-active (SA) particles and gel particles.

trochemical responses (scheme in Figure 6) as: (i) dissolved biopolymer molecules and colloids; (ii) surface-active microparticles, and (iii) gel microparticles. While in the chronoamperometric curve adsorption is manifested as a gradual decrease in the oxygen reduction current, the collision of a particle at the mercury interface leaves a fingerprint on the *I-t* curve of oxygen reduction. Specific electrochemical signals (spikes *vs.* depressions) of the two classes of microparticles enable their simultaneous detection in seawater samples and monitoring of their abundance. On the other hand, the AFM emerges as the method of choice for characterization of marine gels at the nanoscale.[30, 40–42]

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SAŽETAK

Elektrokemijska detekcija mikročestica gela u morskoj vodi

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Prvi puta prikazana je nanostruktura morskog gela oslikana mikroskopijom atomskih sila i nova vrsta elektrokemijskih signala mikročestica gela u morskoj vodi. Čestice gela u morskoj vodi selektivno se detektiraju kao specifični amperometrijski signali primjenom živine kapajuće elektrode kao senzora. Svaki signal je rezultat sudara mikročestice gela s rastućom površinom živine elektrode. Primjenom amperometrijske tehnike i živine kapajuće elektrode kao senzora organske mikročestice detektiraju se na dinamičkoj međupovršini živina elektroda/morska voda temeljem svojstava hidrofobnosti i supramolekulske organizacije kao površinski-aktivne i gel čestice.